REMARKS

Applicants have added into the present specification a substitute paper copy Sequence Listing section according to 37 C.F.R. §1.821(c). Furthermore, attached hereto is a 3 1/2" disk containing the "Sequence Listing" in computer readable form in accordance with 37 C.F.R. §1.821(e).

Applicants have amended the specification to insert SEQ ID Nos, as supported in the present specification.

The following statement is provided to meet the requirements of 37 C.F.R. §1.825(a) and 1.825(b).

I hereby state, in accordance with 37 C.F.R. §1.825(a), that the amendments included in the substitute sheets of the sequence listing are believed to be supported in the application as filed and that the substitute sheets of the sequence listing are not believed to include new matter.

I hereby further state, in accordance with 37 C.F.R. §1.825(b), that the attached copy of the computer readable form is the same as the attached substitute paper copy of the sequence listing.

Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current

amendment. The attached version is captioned "Version with markings to show changes made".

Applicants submit that the present application contains patentable subject matter and therefore urge the examiner to pass the case to issuance.

If the examiner has any questions or comments concerning the above described application, the examiner is urged to contact the undersigned at the phone number below.

The amendments to the claims are made to place the application in better condition for examination.

Favorable consideration is respectfully solicited.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning at page 22, line 11, is replaced with the following rewritten paragraph:

-- The protein having the amino acid sequence represented by SEQ ID NO: 2 is a human type protein (hBSSP4) and the mature type having serine protease activity is the polypeptide represented by the 1st to 268th amino acids. As consensus sequences of serine proteases, it has Ala-Ala-His-Cys ~~represented by the~~ corresponding to the 39th to 42nd amino acid~~s~~ residues of SEQ ID NO:2 and Asp-Ser-Gly-Gly-Pro ~~represented by the~~ corresponding to the 192nd to 196th amino acid~~s~~ residues of SEQ ID NO:2 and one or more of Asp's are present between the concensus sequences. A nucleotide sequence encoding this protein is shown in SEQ ID NO:1.--

The paragraph beginning at the bottom of page 22, is replaced with the following rewritten paragraph:

--The protein having the amino acid sequence represented by SEQ ID NO:4 is a human type protein (hBSSP4) and the mature type having serine protease activity is the polypeptide represented by the 1st to 270th amino acids. As consensus sequences of serine proteases, it has Ala-Ala-His-Cys ~~represented by the~~ corresponding to residues of the 39th to 42nd amino acids of SEQ ID NO:4 and Asp-Ser-Gly-Gly-Pro corresponding to residues of the represented by the 192nd to 196th amino acids of SEQ ID NO:1 and one or more of Asp's are present between the concensus sequences. A nucleotide sequence encoding this protein is shown in SEQ ID NO:3. This

sequence corresponds to SEQ ID NO:1 from which the 943rd to 1217th bases have been removed, and the amino acid sequence ~~represend~~ represented by SEQ ID NO:4 corresponds to the amino acid sequence represented by SEQ ID NO:2 in which the 265th amino acid and the subsequent amino acids are different.--

The paragraph beginning at page 23, line 12, is replaced with the following rewritten paragraph:

--The protein having the amino acid sequence represented by SEQ ID NO:6 is a human type protein (hBSSP4) and the mature type having serine protease activity is the polypeptide represented by the 1st to 257th amino acids. As consensus sequences of serine proteases, it has Ala-Ala-His-Cys ~~represented by the~~ corresponding to the 39th to 42nd amino acid~~s~~ residues of SEQ ID NO:6 and Asp-Ser-Gly-Gly-Pro ~~represented by the~~ corresponding to the 192nd to 196th amino acid~~s~~ residues of SEQ ID NO:2 and one or more of Asp's are present between the concensus sequences. A nucleotide sequence encoding this protein is shown in SEQ ID NO:5. This sequence corresponds to SEQ ID NO:1 from which the 895th to 11208th bases have been removed, and the amino acid sequence represented by SEQ ID NO:6 correspond to the amino acid sequence represented by SEQ ID NO:2 in which the 249th amino acids and the subsequnent amino acids are different. Further, the nucleotide sequence corresponds to the sequence wherein the 969th to 1036th bases of SEQ ID NO:5 are added to the downstream of the 1282 base of SEQ ID NO:1.--

The paragraph beginning at the bottom of page 24, is replaced with the following rewritten paragraph:

--The protein having the amino acid sequence represented by SEQ ID NO: 10 is a human type protein (hBSSP4). As a consensus sequence of serine proteases, this does not have Ala-Ala-His-Cys ~~represented~~ corresponding to the 39th to 42nd amino acid residues of SEQ ID NO:10, but has Asp-Ser-Gly-Gly-Pro ~~represented by the~~ corresponding to residues of the 82nd to 86th amino acids of SEQ ID NO:10. A nucleotide sequence encoding this protein is shown in SEQ ID NO:9. This sequence corresponds to the nucleotide sequence of SEQ ID NO:1 from which the 233rd to 562nd bases have been removed.--

The paragraph beginning at page 24, line 14, is replaced with the following rewritten paragraph:

--The protein having the amino acid sequence represented by SEQ ID NO: 12 is a human type protein (hBSSP4). As a consensus sequence of serine proteases, it has Ala-Ala-His-Cys ~~represented by the~~ corresponding to residues 39th to 42nd amino acids of SEQ ID NO:12 but does not have Asp-Ser-Gly-Gly-Pro corresponding to residues of the 82nd to 86th amino acids of SEQ ID NO:10. A nucleotide sequence encoding this protein is shown in SEQ ID NO:11. This nucleotide sequence corresponds to the nucleotide sequence represented by SEQ ID NO:1 from which the 364th to 562nd amino acids have been removed.--

The paragraph beginning at page 25, line 7, is replaced with the following rewritten paragraph:

--The protein having the amino acid sequence represented by SEQ ID NO:14 is a human type protein (hBSSP4). As a consensus sequence of serine proteases, it has Ala-Ala-His-Cys ~~represented by the~~ corresponding to the 39th to 42nd amino acid~~s~~ residues of SEQ ID NO:14 but do not have Asp-Ser-Gly-Gly-Pro corresponding to residues of the 82nd to 86ht amino acid of SEQ ID NO:10. A nucleotide sequence encoding this protein is shown in SEQ ID NO:13. This nucleotide sequence corresponds to the nucleotide sequence represented by SEQ ID NO:1 from which the 588th to 1145th bases have been removed. There is a possibility that the nucleotide sequence represented by the 652nd and the subsequent bases of SEQ ID NO: 13 would be "ccc ggg ccc cag cgc ttt tgt gta tat aaa tgt taatgatttt tataggtatt tgtaaccctg cccacatatc" SEQ ID NO:49 and the amino acid sequence represented by the 168th and the subsequent amino acids of SEQ ID NO: 14 would be "Pro Gly Pro Gln Arg Phe Cys Val, Tyr Lys Cys" SEQ ID NO:50.

The paragraph beginning at the bottom of page 25, is replaced with the following rewritten paragraph:

--The protein having the amino acid sequence represented by SEQ ID NO:16 is a human type protein (hBSSP4). As a consensus sequence of serine proteases, it has Ala-Ala-His-Cys ~~represented by the~~ corresponding to the 39th to 42nd amino acid~~s~~ residues of SEQ ID NO:16 but does not have Asp-Ser-Gly-Gly-Pro corresponding to the 82nd to 86th amino acid residues of SEQ ID NO:10. A nucleotide sequence encoding this protein is shown in SEQ ID NO:15. This sequence corresponds

to SEQ ID NO: 1 from which the 285th to 562nd bases have been removed.

The paragraph beginning at page 26, line 6, is replaced with the following rewritten paragraph:

--The protein having the amino acid sequence represented by SEQ ID NO: 18 is a human type protein (hBSSP4). As a consensus sequence of serine proteases, it has Ala-Ala-His-Cys ~~represented by the~~ corresponding to the 39th to 42nd amino acid~~s~~ residues of SEQ ID NO:18 but does not have Asp-Ser-Gly-Gly-Pro corresponding to the 82nd to 86th amino acid residues of SEQ ID NO:10. A nucleotide sequence encoding this protein is shown in SEQ ID NO:—17. This sequence corresponds to the sequence wherein the 721st to 948th bases of SEQ ID NO: 17 is added to the downstream of the 720th base of SEQ ID NO: 1, and corresponds SEQ ID NO:1 from which the 720th and the subsequent bases have been removed.--

The paragraph beginning at the bottom page 26, is replaced with the following rewritten paragraph:

--The protein having the amino acid sequence represented by SEQ ID NO:20 is a mouse type protein (mBSSP4) and the mature type having serine protease activity is the polypeptide represented by the 1st to 253 amino acids. As consensus sequences of serine proteases, it has Ala-Ala-His-Cys ~~represented by the~~ corresponding to the 39th to 42nd amino acid~~s~~ residues of SEQ ID NO:20 and Asp-Ser-Gly-Gly-Pro ~~represented by the~~ corresponding to the 192nd to 196th amino acid~~s~~ residues of SEQ ID NO:20 and one or more of Asp's are

present between the concensus sequences. A nucleotide sequence encoding this protein is shown in SEQ ID NO: 19.--

The paragraph beginning at page 35, line 12, is replaced with the following rewritten paragraph:

--The vector is not specifically limited in so far as it can express the protein of the present invention. Examples thereof include pBAD/His, pRSETA, pcDNA2.1, pTrcHis2A, pYES2, pBlueBac4.5, pcDNA3.1 and pSecTag2 manufacture by Invitrogen, pET and pBAC manufactured by Novagen, pGEM manufactured by Promega, pBluescriptII manufactured by Stratagene, pGEX and pUC18/19 manufactured by Pharmacia, PfastBAC1 manufactured by GIBCO and the like. Preferably, a protein expression vector (described in the specification of a patent application entitled "Protein expression vector and its use" and filed by the same applicant on the same day) is used. This expression vector is constructed by using pCRII-TOPO vector described in the Examples hereinafter, or a commercially available expression vector, for example pSecTag2A vector or pSecTag2B vector (Invitrogen) and integrating a secretory signal nucleotide sequence suitable for expression of the protein of the present invention, in the 3' downstream side thereof, a Tag nucleotide sequence, a cleavable nucleotide sequence and a cloning site, into which a nucleotide sequence encoding a target protein can be inserted, in this order. More specifically, it is preferred to use trypsin signal as the secretory signal, a nucleotide sequence encoding polyhistidine as the Tag

nucleotide sequence, and a nucleotide sequence encoding an amino acid sequence which is susceptible to enzyme-specific cleavage, i.e., a nucleotide sequence encoding the amino acid sequence of Asp-Asp-Asp-Asp-Lys SEQ ID NO:51 (said amino acid sequence is recognized by enterokinase, and the recombinant fusion protein is cleaved at the C-terminus part thereof) as the cleavable nucleotide sequence.--

The paragraph beginning at page 58, line 3, is replaced with the following rewritten paragraph:

--The cloning was carried out by PCR using a human brain cDNA library (Clontech) as a template and nucleotide sequences corresponding to an amino acid sequence common to serine proteases represented by

Primer 1: GTG CTC ACN GCN GCB CAY TG (SEQ ID NO: 30)

Primer 2: CCV CTR WSD CCN CCN GGC GA (SEQ ID NO: 31)

as primers. Namely, 5 µl of the template, 5 µl of 10 x ExTaq buffer, 5 µl of dNTP, 10 pmol of each of the above primers and 0.5 µl of ExTaq (TAKARA) were added and the total volume was adjusted to 50 µl with sterilized water. PCR was carried out by repeating a cycle of heating at 94°C for 0.5 minute, at 55°C for 0.5 minute and then at 72°C for 1 minutes, 35 times. The PCR product was mixed with pCR II-TOPO vector attached to TOPO TA cloning kit (Invitrogen) and the mixture was allowed to stand at room temperature for 5 minutes. Then, according to a conventional manner, *E. coli* Top 10 attached to the kit was transformed and applied to a LB (Amp^+) plate (containing 100 µg/ml of ampicillin). According to a conventional manner,

a plasmid was extracted from each colony obtained and its nucleotide sequence was determined by cycle sequencing method with a fluorescence sequencer (ABI). Homology of the sequence of each clone was examined by means of GenBank. Regarding an unknown sequence, i.e., BSSP4 gene, the full length cDNA was obtained by 5' RACE and 3' RACE and, according to the same manner as described above, the nucleotide sequence was determined. Namely, BSSP4 clone specific primers, GSP1 primers [hBSSP4F1 (SEQ ID NO: 32) or hBSSP4R1 (SEQ ID NO: 36)] and GSP2 primers [hBSSP4F2 (SEQ ID NO: 33) or hBSSP4R2 (SEQ ID NO: 37)] were prepared. PCR was carried out by using human brain Marathon-Ready cDNA (Clontech), AP1 primer attached to this reagent and either of the above GSP1 primers and heating at 94°C for 2 minutes once and repeating a cycle of heating at 94°C for 30 seconds, at 60°C for 30 seconds and then at 72°C for 30 seconds 35 times. Then, 5 µl of the PCR product diluted to 1/100, 5 µl of 10 x buffer, 5 µl of dNTP, 10 pmol of either of 10 µM of the above GSP2 primer, 10 pmol of AP2 primer attached to the above reagent and 0.5 unit of ExTaq were admixed and adjusted to 50 µl with sterilized water. Then, according to the same manner as the above, PCR was carried out. The PCR product was cloned by the above TOPO TA cloning kit and sequenced to obtain the upstream and downstream regions of the above clone. At this time, as for a clone which seemed not to cover the full length of a protein, the specific primers shown hereinafter were prepared based on the newly found nucleotide sequence. Further, based on this sequence, the primers capable of amplifying ORF as shown

hereinafter [hBSSP4F6 (SEQ ID NO: 35) and hBSSP4R3/E (SEQ ID NO: 38) or hBSSP4R4/E (SEQ ID NO: 39)] were prepared and PCR carried out using human brain Marathon-ready cDNA as a template to confirm that these clones were identical. This was cloned into pCR II-TOPO vector attached to TOPO TA cloning kit to obtain the plasmid pCR II/hBSSP4 containing the full length cDNA clone. The nucleotide sequence of DNA contained in this plasmid is shown in SEQ ID NO: 1 and the amino acid sequence of hBSSP4 protein deduced from the nucleotide sequence is shown in SEQ ID NO: 2. Further, two different types of clones were obtained. The amino acid sequence of hBSSP4 represented by SEQ ID NO: 2 (the 1st to 268th amino acids) is hBSSP4 mature or active type protein composed of 268 amino acids. In the amino acid sequence represented by SEQ ID NO: 2, the -49th to -1st amino acids are a prepro or pro part and the -15th to -1st amino acids are a pro part and are considered to be a precursor of hBSSP4. As consensus sequences of serine proteases, there are Ala-Ala-His-Cys represented by the 39th to 42nd amino acids~~s~~ residues of SEQ ID NO:2 and Asp-Ser-Gly-Gly-Pro represented by the 192nd to 196th amino acids~~s~~ residues of SEQ ID NO:2 and there are one or more Asp's between these consensus sequences.--

The paragraph beginning at page 61, line 10, is replaced with the following rewritten paragraph:

--According to the same manner, 5' RACE and 3' RACE were carried out by using the primers as described hereinafter and mouse brain Marathon-Ready cDNA (Clontech) as a template,

followed by cloning to obtain mouse homologous gene pCRII/mBSSP4. The nucleotide of DNA containing this plasmid is shown by SEQ ID NO:19 and the amino acid sequence of mBSSP4 protein deduced from this nucleotide sequence is shown in SEQ ID NO:20. The amino acid sequence of mBSSP4 represented by SEQ ID NO:20 (the 1st to 259th amino acids) is mBSSP4 mature or active type protein composed of 259 amino acids. In the amino acid sequence represented by SEQ ID NO:20, the -49th to 1st amino acids are a prepro or pro part and the -15th to -1st amino acids are a pro part and are considered to be a precursor of mBSSP4. As consensus sequences of serine proteases, there are Ala-Ala-His-Cys (the 39th to 42nd amino acids residues of SEQ ID NO:20) and Asp-Ser-Gly-Gly-Pro (the 192nd to 196th amino acids residues of SEQ ID NO:20) and there are one or more Asp's between the consensus sequences.

human BSSP4

hBSSP4F1	Forward	AGGTTCCTATCATCGACTCG	RACE (SEQ ID NO: 32)
hBSSP4F2	Forward	TGAGGACATGCTGTGTGCCGG	RACE (SEQ ID NO: 33)
hBSSP4F3	Forward	GTTGTGGGCGGCGAGGACAG	mature (SEQ ID NO: 34)
hBSSP4F6	Forward	GCCATGGTGGTTTCTGGAGC	FL* (SEQ ID NO: 35)
hBSSP4R1	Reverse	TATGGTTTGTTCAGGTTGTCC	RACE (SEQ ID NO: 36)
hBSSP4R2	Reverse	AGGGCAATGTCTGCACAGGC	RACE (SEQ ID NO: 37)

hBSSP4R3/E	Reverse	CTGAATTCCTAGGAGCGCGCGGCGGCC	FL*
			(SEQ ID NO: 38)
hBSSP4R4/E	Reverse	GAGAATTCGATATGTGGGCAGGGTTACA	FL*
			(SEQ ID NO: 39)
mouse BSSR4			
mBSSP4.1	Forward	ACAAACCATCTCTGTTCTCAG	RACE
			(SEQ ID NO: 40)
mBSSP4F2	Forward	GTCCCAGAAAGTAGGCATTG	RACE
			(SEQ ID NO: 41)
mBSSP4F3	Forward	CTCCACCCATACCAGCAATG	FL*
			(SEQ ID NO: 42)
mBSSP4F4	Forward	ATTGTGGGAGGTGAGGACAG	mature
			(SEQ ID NO: 43)
mBSSP4.2	Reverse	TGCAGAGTTCGGAGTCGATG	RACE
			(SEQ ID NO: 44)
mBSSP4R2	Reverse	ATCCAGCAGTCGGTCTTGGG	RACE
			(SEQ ID NO: 45)
mBSSP4R3/P	Reverse	ATTCTGCAGTTCCTTGTTCTCTCGCTCAGG	FL*
			(SEQ ID NO: 46)

*: for full length

The paragraph beginning at the bottom of page 68, line 19, is replaced with the following rewritten paragraph:

--Amplification was carried out by using the primers having the sequences represented by SEQ ID NOS: 25 and 26 so that the peptide of Leu-Val-His-Gly SEQ ID NO:52 was present at the C-terminus of the part from trypsin signal to the enterokinase recognition site of pSecTrypHis/neurosin. This

was inserted between NheI and HindIII sites of pSecTag2A to construct the plasmid pTrypSig.--

In the Claims:

Claims 55-57, 66-69, and 71 have been amended as follows below:

55(Twice-Amended). A vector comprising the nucleotide sequence according to claim ~~2~~ 77.

56(Twice-Amended). Transformed cells having the nucleotide sequence according to ~~2~~ 77 in an expressible state.

57(Twice-Amended). A process for producing a protein which comprises culturing cells transformed with the nucleotide sequence according to (i) to (xxxiii), (xliii) to (lx) or (lxiv) to (lxxxvi) of claim ~~2~~ 77 or a fragment thereof, and collecting hBSSP4 produced.

64(Twice-Amended). An antibody against the protein according to claim ~~1~~76 or a fragment thereof.

66(Twice-Amended). A process for producing a monoclonal antibody against the protein according to claim ~~1~~76 or a fragment thereof which comprises administering the protein according to claim ~~1~~76 or a fragment thereof to a warm-blooded animal other than a human being, selecting the animal whose antibody titer is recognized, collecting its spleen or lymph node, fusing the antibody producing cells

contained therein with myeloma cells to prepare a monoclonal antibody producing hybridoma.

67(Twice-Amended). A method for determining the protein according to claim ~~1~~76 or a fragment thereof in a specimen which is based on immunological binding of an antibody against the protein or a fragment thereof to the protein or a fragment thereof.

68(Twice-Amended). A method for determining hBSSP4 or a fragment thereof in a specimen which comprises reacting a monoclonal antibody or a polyclonal antibody against the protein (a) to (v) or (cc) to (nn) of claim ~~1~~76 or a modified derivative or fragment thereof and a labeled antibody with hBSSP4 or a fragment thereof in the specimen to detect a sandwich complex produced.

69(Twice-Amended). A method for determining hBSSP4 or a fragment thereof in a specimen which comprises reacting a monoclonal antibody or a polyclonal antibody against the protein (a) to (v) or (cc) to (nn) of claim ~~1~~76 or a modified derivative or fragment thereof or a fragment thereof with labeled hBBSP4 and hBSSP4 or a fragment thereof in the specimen competitively to detect an amount of hBSSP4 or a fragment thereof in the specimen based on an amount of the labeled hBBSP4 reacted with the antibody.

71(Twice-Amended). A diagnostic marker for diseases

in tissues comprising the protein according to claim ~~1~~76.